

MSU 4.1-672
Appl. No. 10/725,214
Amendment dated September 22, 2006
Reply to Office Action of July 25, 2006

SPECIFICATION

Please replace paragraph [0045] on pages 15 and 16 with the following amended paragraph [0045].

[0045] **MTT cell proliferation assay:** The cells were counted and transferred to 96 well microtiter plates, and incubated for 24 h prior to the addition of test compounds. The cell numbers used for each cancer cell lines were 6000, 3000, 4000, 3000 and 5000 per well for SF-268, NCI H460, MCF-7, HCT-116, and AGS, respectively. Test compounds were dissolved in DMSO and diluted with sterile RPMI-1640 media as necessary to obtain the appropriate concentration. The test solutions were then added to the wells containing cells in 100- μ L aliquots to obtain final appropriate concentrations. The final concentration of DMSO in each well was 0.2%. Test compounds, positive control, and blank control (DMSO in media) were incubated with all five cell-lines for 48 h, after which MTT solution (5 mg/mL in PBS solution) was added into each well in 25- μ L aliquots. The plates then were wrapped in aluminum foil and incubated for three hours at 37°C with 5% CO₂ and 80% RH. The RPMI media, MTT

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and floating cells from each well were removed and aliquots of DMSO (20 μ L) added into each sample well to dissolve the purple formazan crystals. The plates were then shaken for eight minutes on a gyrorotary shaker after which the absorbence of the contents of each well was recorded with an automated microplate reader (model EL800, Bio-Tek Instruments, Inc., Winooski, VT) at 570 nm. The experiments were performed in triplicate at concentrations of 25, 50, 100 and 200 μ g/mL. Cell viability was determined by comparing the average absorbance of three test wells verses that of the blank control wells. Results are expressed in a line graph as the percentage of cell viability against concentration of compounds in Figures ~~6 to 10~~ 5 to 7.